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Interactions of Divalent Metal Ions with Inorganic and Nucleoside Phosphates. 7. Kinetics of the Ni(II)-P_i, -RibP, and -CMP Systems

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Temperature-jump kinetic measurements were carried out at 15 °C and $I = 0.1$ in Ni²⁺ solutions of inorganic, ribose, and cytidine monophosphates. Relaxation times in the 10⁻⁴-s time range showed a very strong pH as well as concentration dependence. The results were consistent with a complexation mechanism involving protonated (HL⁻) and unprotonated (L²⁻) ligands. Rate constants for the reactions of Ni²⁺ with L²⁻ and HL⁻ were about $1.5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $3.2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, respectively, for all three systems. Ligand penetration rate constants were approximately equal to the Ni²⁺ solvent-exchange rate at that temperature.

In previous papers^{1,2} in this series, we reported on the kinetics of inorganic and nucleoside di- and triphosphate interactions with Ni²⁺ and Mg²⁺. For these two metal ions and the inorganic phosphates, a mechanism was found that involved metal complexes with the monoprotonated as well as the unprotonated ligand. For the metal ion-nucleotide systems, the relaxation spectra included kinetic pathways involving the formation of 2:1 complexes such as Mg₂-ATP and Ni₂-ATP. In all cases, the complexation steps were consistent with the Eigen-Tamm mechanism wherein the rate-determining step is the expulsion of one or more water molecules from the inner coordination sphere of the metal ion.

We now turn our attention to the corresponding monophosphate systems, including inorganic phosphate, ribose phosphate, and nucleotides. Although transition metal ions such as Ni²⁺ can interact with the nitrogens in the nucleoside portion of the molecule, the principal interaction occurs with the phosphate group. As a consequence, it will be useful to characterize the kinetics of the phosphate interactions alone before carrying out kinetic studies with the nucleotides. CMP is included in the present report because of its similarity in behavior to the phosphates. The only prior work in Ni²⁺-monophosphate kinetics is that reported some years ago by Brintzinger and Hammes³ on the Ni-methyl phosphate system. The kinetic profiles for the adenine nucleotides are considerably more complicated. These systems are reported in the following paper.⁴

Experimental Section

Materials. Ribose phosphate (RibP) and cytidine phosphate (5'-CMP) were purchased from Sigma. HPO₄²⁻ was prepared from either KH₂PO₄ (Matheson Coleman and Bell) or reagent phosphoric acid (Fisher). All solutions were prepared daily. Stock solutions of Ni²⁺ were prepared from reagent grade nitrate (Baker) and standardized by EDTA titration.

Methods. All kinetic experiments were carried out at 15 °C and $I = 0.1 \text{ M}$ (KNO₃) on a temperature-jump spectrometer⁵ (Messan-lagan Studiengesellschaft). A precooled solution was placed in the T-jump cell and equilibrated at the initial temperature (10 °C) in the T-jump apparatus. The kinetic experiment was carried out by discharging 35 kV through the system, thus raising the temperature to 15 °C. The relaxation effect, monitored by changes in transmittance of a pH indicator (chlorophenol red, pK_{In} = 6.0), was stored on a Biomation 802 transient recorder, displayed on an oscilloscope screen, and analyzed for the relaxation time. At least two T-jump deter-

Table I. Stability Constants and pK_a Values for the Monophosphates^a

protonated ligand	pK _{a1}	pK _{a2} (phosphate)	complex	K _{ML}	K _{MHL} ^e
H ₂ (RibP)	1.4 ^c	6.22	Ni-RibP	80	5
H ₂ (P _i) ^b	2.0 ^c	6.79	Ni-P _i	100	3
H ₂ (5'-CMP)	4.52 ^d	6.27	Ni-5'-CMP	80	3

^a At 15 °C and $I = 0.1$ (KNO₃); from ref 7 unless otherwise indicated. ^b L = HPO₄, HL = H₂PO₄. ^c Phosphate ionization. ^d Nucleoside base ionization. ^e This work.

minations were carried out for each solution. The pH of the test solution was measured, in a vessel thermostated at 15 °C, by a Corning semimicro combination electrode in conjunction with a Corning 101 digital electrometer. The pH was adjusted by adding 1 or 0.1 M KOH or HNO₃ solution from a 50-μL syringe.

The concentrations of ligand and metal ion ranged from 3×10^{-3} to $2 \times 10^{-2} \text{ M}$, and the ligand:metal ratio was near unity in most cases. The concentration of the indicator was ca. $4 \times 10^{-5} \text{ M}$. Reactions were followed at 530-570 nm, depending on the pH and concentration of the solution studied. Blank solutions, consisting of metal and indicator or ligand and indicator, displayed only rapid proton-transfer effects. Slight variations in ionic strength due to varying amounts of reactant ions did not cause significant changes in the kinetic effects.

pK_a values and stability constants for the NiL and NiHL (MHL) systems were determined by potentiometric titration, as described earlier.⁶ Results were analyzed by a nonlinear regression procedure.^{6b} The pK_{a2} for inorganic phosphate was somewhat higher than those for the ribose phosphate and CMP systems. Stability constants for complexes formed with Ni²⁺ were very similar among the three systems and probably were the same within experimental error.

Treatment of Data. Relaxation times were determined either from log amplitude vs. time plots or by matching the experimental traces with a series of standard relaxation curves of known τ values on transparent overlays. Either procedure enabled us to determine relaxation times to a precision of 5% or better. Table I lists equilibrium constants for the systems studied. These values, together with the overall metal ion and ligand concentrations and the pH, were used to calculate equilibrium concentrations of the species present.

The evaluation of K_{os}, the outer-sphere equilibrium constant shown in reaction B, has been described elsewhere.^{7,8} For our experimental conditions we estimate K_{os} to be 10 M⁻¹ for a 2:2 charge interaction and K_{os} = 2 M⁻¹ for a 2:1 charge interaction.

Results

Kinetic data for the systems Ni(II)-HPO₄, Ni(II)-RiPO₄, and Ni(II)-5'-CMP were obtained at 15 °C in 0.1 M KNO₃ over the pH range 5.0-6.8. Several concentrations were used

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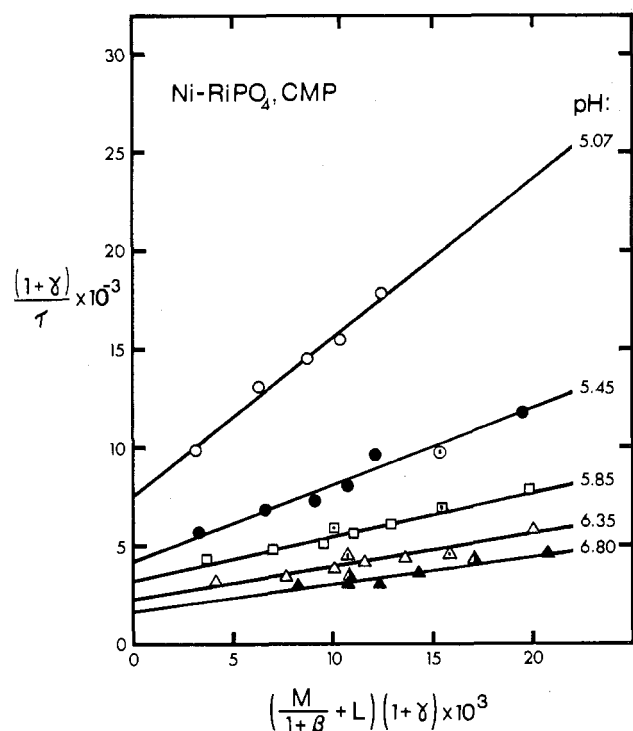


Figure 1. Concentration and pH dependence of the relaxation time for the Ni-RibP and -CMP systems. Solid lines are calculated from eq 1 with the constants in Table III, and the Ni-CMP system is designated by the following: \circ , \square , ∇ , ∇ .

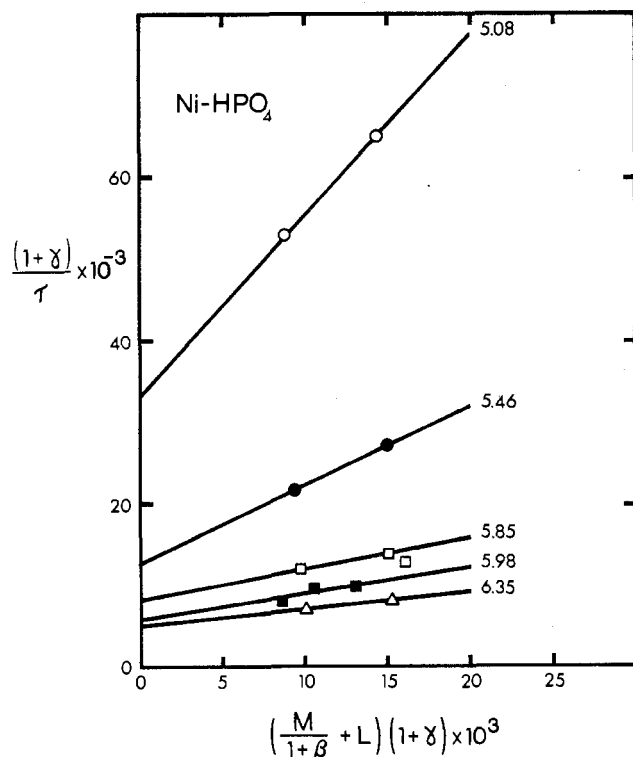


Figure 2. Concentration and pH dependence of the relaxation time for the Ni-P_i system. Solid lines are calculated from eq 1 with the constants in Table III.

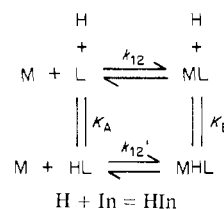
at each pH. A single relaxation effect, in the region of ca. 0.1 ms, was observed (in addition to the rapid proton transfer effects, which were not studied in detail). The relaxation times were found to be both concentration and pH dependent (Table II and Figures 1 and 2). The pH dependence is much stronger than that predicted for an association mechanism involving

Table II. Kinetic Data for Various Nickel Phosphate Systems^a

$10^2[M^0], M$	$10^2[L^0], M$	pH ^b	τ^{-1}, s^{-1}
1. Ni-RibP			
2.0	2.0	6.79	4440
		6.36	5260
		5.86	5800
		5.45	6250
		5.04	7690
1.2	1.2	6.85	3510
		6.37	4040
		5.85	4650
		5.45	5560
		5.06	6150
1.0	0.98	6.81	3180
		6.31	3850
		5.87	4440
		5.45	4760
		5.08	5710
0.84	0.84	6.79	2980
		6.33	3510
		5.86	4040
		5.46	4350
		5.06	5400
0.60	0.60	6.79	2940
		6.34	3390
		5.86	3920
		5.45	4260
		5.07	5260
0.30	0.30	6.35	3030
		5.86	3570
		5.45	3760
		5.07	4350
		2. Ni-P _i	
1.202	2.91	5.88	6670
1.20	1.16	6.35	5560
		5.85	6900
		5.46	8500
		5.08	11100
		1.02	1.0
0.80	1.01	5.96	5460
0.64	0.81	6.00	4860
0.75	0.754	5.08	10500
		5.46	7690
		5.85	6350
		6.35	5000
		3. Ni-5'-CMP	
0.928	1.84	6.12	5180
0.496	0.491	6.46	3120
1.00	0.803	6.78	3390
		6.35	4080
		5.85	4650
		5.45	5260
		6.81	4050
1.50	1.50	6.35	4440
		5.84	5560
		5.50	6140

^a At 15 °C, $I = 0.1 M KNO_3$. ^b pH values were converted to C_H by $\gamma_H = 0.83$.

Scheme I



solely the free ligand L. In fact, the dependence of $1/\tau$ upon pH (Figures 1 and 2) goes in the wrong direction for such a mechanism: the τ^{-1} values are *higher* at lower pH. This behavior is suggestive of, and can be quantitatively accounted for by, the addition of a parallel reaction pathway $M + HL$

Table III. Summary of Rate Constants for Ni-Monophosphate Interactions^a

constant	ligand		
	RibP	P _i	5'-CMP
10 ⁻⁵ k ₁₂ ^b , M ⁻¹ s ⁻¹	1.40 ± 0.03	1.81 ± 0.16	1.40 ± 0.20
10 ⁻⁴ k ₁₂ ^b , M ⁻¹ s ⁻¹	3.30 ± 0.10	3.31 ± 0.15	3.16 ± 0.97
10 ⁻³ k ₂₁ ^b , s ⁻¹	1.75 ± 0.04	1.81 ± 0.16	1.75 ± 0.25
10 ⁻³ k ₂₁ ^b , s ⁻¹	6.6 ± 0.2	11 ± 0.5	10 ± 3
10 ⁻⁴ k _{1p} ^c	1.4	1.8	1.4
10 ⁻⁴ k _{1p} ^c	1.6	1.6	1.6

^a At 15 °C and 0.1 M KNO₃. ^b Values obtained from minimization procedure. ^c Calculated from the relationship $k_{\text{obsd}} = K_{\text{os}}k_{1p}$. At 15 °C and $I = 0.1$ M KNO₃, $K_{\text{os}} = 2 \text{ M}^{-1}$ for the 2:2 and 2:1 charge interactions, respectively.

⇌ MHL to the pathway $\text{M} + \text{L} \rightleftharpoons \text{ML}$, where HL is the protonated ligand. (For the inorganic phosphate $\text{L} = \text{HPO}_4^{2-}$, $\text{HL} = \text{H}_2\text{PO}_4^-$.) This mechanism is shown as Scheme I. The rapid proton transfer reactions involving HL, HIn, and MLH are treated as preequilibria. For the parallel slow steps, designated by the arrows, there is a single relaxation time given by

$$\frac{1}{\tau} = (k_{12} + k_{12}'K_A[\text{H}])\left(\frac{[\text{M}]}{1 + \beta} + [\text{L}]\right) + (k_{21} + k_{21}'K_B[\text{H}])\left(\frac{1}{1 + \gamma}\right) \quad (1)$$

where

$$\beta = \frac{K_A[\text{H}][1 + K_B([\text{H}] + ([\text{ML}] + [\text{L}])/(1 + \alpha))]}{1 + K_A([\text{L}]/(1 + \alpha) + K_B([\text{H}] + [\text{ML}]/(1 + \alpha))}$$

$$\gamma = \frac{K_B[\text{H}][1 + K_A([\text{H}] + ([\text{ML}] + [\text{L}])/(1 + \alpha))]}{1 + K_A([\text{L}]/(1 + \alpha) + [\text{H}]) + K_B([\text{ML}]/(1 + \alpha))}$$

$$\alpha = [\text{In}]/([\text{H}] + K_{\text{In}}) \quad K_A = [\text{HL}]/([\text{H}][\text{L}])$$

$$K_B = [\text{MHL}]/([\text{ML}][\text{H}]) \quad K_{\text{In}} = [\text{In}][\text{H}]/[\text{HIn}]$$

The expression for τ^{-1} , eq 1, involves rate constants for the two pathways. Let us define the effective forward rate constant k_{1f} :

$$k_{1f} = k_{12} + k_{12}'K_A[\text{H}] \quad (2)$$

At a single pH the value of k_{1f} is constant, and a plot of $\tau^{-1}(1 + \gamma)$ vs. $([\text{M}]/(1 + \beta) + [\text{L}])/(1 + \gamma) = f(C)$ will generate a family of straight lines with different slopes, increasing as the pH decreases, as shown in Figures 1 and 2. The intercepts of these plots are the effective reverse rate constants k_{1r} ($k_{1r} = k_{21} + k_{21}'K_B[\text{H}]$) at the corresponding pH values.

The term $f(C)$ is a function of equilibrium constants and equilibrium concentrations, which are either known or can be calculated. The terms β and γ are numerically very similar and range from 0.03 at high pH to 2.5 at the lowest pH. The small values of β and γ above pH 6.3 correspond to the virtual disappearance of the protonated pathway above that pH. K_B is given by the identity $K_B \equiv (K_{12}'/K_{12})K_A$. As a consequence, only the two rate constants k_{12} and k_{12}' remain to be determined. Best fit values of those constants and their standard deviations were obtained from a least-squares minimization analysis of all the data points via eq 1. The results are shown in Table III. The solid lines in Figures 1 and 2 were calculated with these constants.

Alternatively, the k_{12} and k_{12}' values can be evaluated by a graphical procedure. The experimental data at different pH values were treated separately; k_{1f} values for each pH were obtained from the slopes of $(1 + \gamma)/\tau$ vs. $f(C)$ plots (Figures 1 and 2). By plotting these k_{1f} values against $K_A[\text{H}]$, we can obtain k_{12} and k_{12}' from the intercept and slope, respectively

(Figure 3). The rate constants calculated by the graphical method are in good agreement with those obtained from the minimization procedure, as can be seen by comparison of Figure 3 and Table III. Both methods were used for each system in this study; the regression method is statistically superior, while the graphical procedure is easier to visualize.

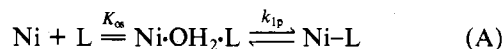
Ni-RibP. The kinetics of Ni(II)-5'-RibP were studied at five different pH values: 5.06, 5.45, 5.85, 6.35, 6.80. The overall Ni(II) and RibP concentrations ranged from 3×10^{-3} to 2×10^{-2} M with the $[\text{M}^0]:[\text{L}^0]$ ratio near unity. Under comparable conditions, our $1/\tau$ values for similar concentrations and pH are in agreement with the values obtained by Brintzinger and Hammes³ for the Ni-MePO₄ interaction, when one takes into account the difference in temperature between the two studies. Quantitative comparisons are difficult because the concentrations used in their work were much higher and because they did not carry out a pH dependence of the relaxation time. $(1 + \gamma)/\tau$ values for RibP are plotted against $f(C)$ in Figure 1. As can be seen in the graph, $(1 + \gamma)/\tau$ varies linearly with $f(C)$ at any single pH and increases as the pH decreases as predicted for Scheme I. The k_{12} , k_{12}' values for this system that best describe the data at all the pH values, obtained from the computer minimization procedure, are $(1.40 \pm 0.03) \times 10^5$ and $(3.30 \pm 0.10) \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, respectively.

Ni-P_i. The kinetic behavior of the Ni-P_i system was found to parallel that of Ni(II)-RibP, with τ^{-1} values higher at lower pH. At the same $[\text{M}^0]$, $[\text{L}^0]$, and pH, τ^{-1} values for Ni-P_i are about 50% higher than those for Ni-RibP. The data can be fitted to eq 1, and k_{12} , k_{12}' obtained by the same computer fitting procedure are $(1.81 \pm 0.16) \times 10^5$ and $(3.31 \pm 0.15) \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$. It is obvious that τ^{-1} for Ni-P_i should be higher than Ni-RibP at the same $f(C)$ and pH, since the constant K_A is 4 times that of RibP. In an earlier report,⁸ before the strong pH dependence of τ^{-1} was observed, we were hard pressed to offer an explanation for the difference between RibP and P_i. The kinetic data for this system are shown in Table II, and the results are plotted in Figure 2.

Ni-5'-CMP. The kinetics of Ni(II) complexation with this nucleotide are included in this report due to similarities with the ribose phosphate system. The stability constant of 5'-CMP-Ni(II) is identical with that of Ni(II)-RibP (Table I), and its kinetic results also coincide with those of Ni(II)-RibP. The kinetic data are shown in Table II. The τ^{-1} values can be reproduced from eq 1 by using the same k_{12} , k_{12}' values as RibP, within experimental error. k_{12} , k_{12}' obtained by analyzing the data in terms of eq 1 are shown in Table III.

Discussion

Rate constants obtained for the three systems are summarized in Table III. In addition, we have calculated k_{1p} values for the two-step ligand substitution mechanism generally recognized for Ni(II) complexes:⁹⁻¹¹



for which

$$k_{\text{obsd}} = K_{\text{os}}k_{1p} \quad (3)$$

where k_{obsd} would be equivalent to our k_{12} and k_{12}' for the L and HL pathways. Values of K_{os} used in calculating k_{1p} are 10 and 2 for L²⁻ and HL⁻ reactions, respectively, as mentioned earlier. Values of k_{1p} calculated for the three ligands, both L²⁻ and HL⁻, all agree with the water-exchange rate constant¹²

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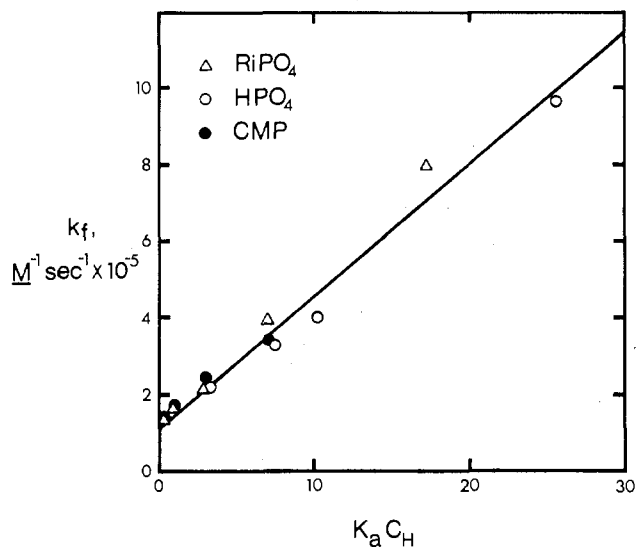


Figure 3. Dependence of the apparent rate constant, eq 2, on the hydrogen ion concentration for the Ni-RibP, -P_i, and -CMP systems.

for Ni(II) under these conditions. This is strong evidence that Scheme I is indeed the correct one.

The values for k_{12} and k_{12}' for the three systems are the same within experimental error. Since the only possible binding sites for orthophosphate or ribose phosphate are the phosphate oxygens, the results indicate that 5'-CMP, despite the presence of the basic nitrogen in its cytosine group, interacts with Ni(II) through the phosphate group only.¹³ This result is contrary to what has been observed for Ni(II) interactions with the adenine nucleotides.¹³⁻¹⁵ Though back-

bound complexes have been shown to exist between Ni(II) with other nucleotides,² the N₃ nitrogen in 5'-CMP apparently does not interact with Ni(II), probably for steric reasons. That Ni(II) binds with 5'-CMP only at the phosphate position is also reflected in its binding constant K_{ML} (80 M), which is the same as that for Ni-RibP.

The reverse rate constants k_{21} , k_{21}' are an indication of the bond strength. The similarity of k_{21} , k_{21}' values among the systems is further evidence of the bond being of the same type, i.e., Ni-O interaction. The agreement of the 5'-CMP values with the other two phosphates again shows the noninvolvement of the cytosine group in the complex formation. The higher value of k_{21}' compared to k_{21} (over fivefold) reflects the increased bond strength for the more highly charged species and suggests the possibility of chelation in the deprotonated ligand L^{2-} .

The relatively precise results obtained for the Ni(II)-RibP system can be utilized to estimate the forward rate constants for phosphate interactions in other Ni(II)-mononucleotide systems. At any pH k_{1f} can be predicted from the equation $k_{1f} = (1.40 \times 10^5) + (3.3 \times 10^4)K_A[H^+]$ (cf. Figure 3). If the pH is kept sufficiently high ($K_A[H^+] < 0.5$) the second term becomes negligible and only the L^{2-} pathway is observed. Due to solubility and other experimental limitations, one often has to carry out measurements at a lower pH, and the second term involving the HL^- pathway has to be included.

In conclusion, we have shown that Ni(II) binds both the monoprotonated and deprotonated phosphate groups of the ortho, 5'-ribose, and cytidine monophosphates, via the dissociative mechanism common to octahedral metal ions. The cytidine ring appears to exert no effect on the binding of Ni^{2+} to CMP.

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Registry No. ribose phosphate, 4300-28-1; 5'-cytidine phosphate, 63-37-6; HPO_4^{2-} , 14066-19-4; Ni^{2+} , 14701-22-5.

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